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RESEARCH ARTICLE

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Amlodipine: Can act as an antioxidant in patients with transfusion-dependent β-thalassemia? A double-blind, controlled, crossover trial

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Abstract

Background and Aim: This study aimed to assess the antioxidant effects of amlodipine in transfusion-dependent β-thalassemia (TDT) patients.

Methods: This crossover trial consisted of two sequences (AP and PA). In the AP sequence, nine cases received amlodipine 5 mg daily (phase I) and then were switched to placebo (phase II). In PA sequence, 10 patients took the placebo (phase I) and were shifted to amlodipine (phase II). The washout period was 2 weeks. The length of each phase was 6 months. Serum malondialdehyde (MDA, μmol/L), carbonyl (protein CO, μM/L), glutathione (GSH, nM/L), and total antioxidant capacity (TAC, μmol FeSO4/L) were measured in the beginning and at the end of phases I and II. The clinical significance was viewed as a minimum change difference of 5% for each outcome between amlodipine and placebo.

Results: Seventeen cases completed the study. According to the baseline MDA values, the adjusted Hedges's g for MDA was −0.59, 95% confidence interval [CI] −1.26 to 0.08. After controlling the baseline protein CO values, Hedges's g computed for protein CO was −0.11, 95% CI −0.76 to 0.55. The estimated values of the adjusted Hedges's g for GSH and TAC were also 0.26, 95% CI −0.40 to 0.91, and 0.42, 95% CI −0.24 to 1.09, respectively. The change difference for MDA was 8.3% (protein CO 2.2%, GSH 3.1%, and TAC 12.9%).

Conclusion: Clinically, amlodipine therapy is an efficacious adjuvant treatment with conventional iron chelators for improving the levels of MDA and TAC in patients with TDT.

KEYWORDS
amlodipine, malondialdehyde, oxidative stress, protein carbonyl, reactive oxygen species, red-cell transfusion, β-thalassemia

1 | INTRODUCTION

Thalassemia is well-known as the most prevailing hemoglobinopathy disorder globally.[1] Increased iron absorption and constant red-cell transfusions bring about iron overload in patients with transfusion-dependent β-thalassemia (TDT).[2,3] Iron accumulation produces reactive oxygen species (ROS) through the Haber-Weiss and Fenton reactions in conjunction with the production of highly toxic hydroxyl radicals due to peroxidation.[4] ROS can damage lipids, proteins, deoxyribonucleic acid (DNA), and intracellular organelles, e.g., lysosomes and mitochondria.[5] This injury can lead to cellular dysfunction, apoptosis, and necrosis, leading to toxicity and dysfunction in the target organs.[6,7] Hydroxyl radical production and increased lipid peroxidation would be determining factors to trigger cardiac problems caused by iron surplus.[8–10]

Various studies have shown that calcium channels are involved in iron absorption by myocardial cells. L-type calcium channel blockers (L-TCC blockers), e.g., amlodipine, are traditionally considered therapeutic options in arrhythmia and hypertension, which can inhibit calcium influx into the cell. According to studies, L-TCC blockers may reduce cellular iron uptake and oxidative stress, preventing iron damage.[11] Recent research has recommended amlodipine prescription to mitigate iron deposition into the tissues and organs, such as cardiac in TDT patients.[12,13]

Amlodipine could reduce oxidative stress in the cardiac and blood in cholesterol-fed rabbits and modify lipid levels.[14,15] Moreover, a clinical trial comprising diabetic patients under treatment with amlodipine could effectively decrease oxidative stress markers.[16] Although some studies indicate the antioxidant effects of amlodipine, few trials have been executed in the thalassemia population. As raised oxidative stress exacerbates the disease complications, exploring and determining the antioxidant properties of amlodipine in these cases can contribute to whether amlodipine can efficiently alleviate oxidative stress and even the severity of complications in the long run. Therefore, this study aimed to evaluate the antioxidant effects of amlodipine in TDT cases.

2 | MATERIALS AND METHODS

2.1 | Ethics considerations

The study protocol and off-label use of the drug were approved by the Institutional Review Board (IRB) of Mazandaran University of Medical Science (MAZUMS). This study was registered in the Iranian Registry of Clinical Trials (IRCT; IRCT20090613002027N16, https://en.irct.ir/trial/33008). The current research adhered to the guidance in the Declaration of Helsinki, and all cases gave written, informed consent before enrollment. Patients were also aware of the study's results at the end of the trial.

2.2 | Study population

In this randomized, double-blind, crossover trial, 19 patients were recruited from the thalassemia ward of Bu-Ali Sina Hospital. Cases with myocardial T2*-weighted magnetic resonance imaging (MRI T2*) less than 14 ms – as moderate or severe cardiac siderosis[17] – and who were over 18 years old without a history of hypertension were eligible for inclusion in the trial if they were also with β-thalassemia major under regular transfusion (transfusion-dependent cases defined as 1–2 RBC units over 4 weeks with no transfusion-free period >35 days). The use of any other types of calcium channel blocker (CCB) and antioxidants, e.g., vitamin C and E, and any changes in type and dose of iron chelators over the trial were considered the exclusion criteria.

2.3 | Study design and intervention

The study was composed of two sequences (AP and PA). In the AP sequence, nine cases received amlodipine 5 mg daily (phase I) and then were switched to placebo (phase II). In the PA sequence, 10 cases took the placebo (phase I) and were shifted to amlodipine (phase II). The washout period was 2 weeks. The length of each phase was 6 months. This study was conducted from June 2018 to August 2019. The drugs were prepared by SOBHAN company, Iran (AMLODIPINE-SOBHAN).

2.4 | Blinding and random allocation

The study was set up at the Thalassemia Research Center (TRC), Mazandaran University of Medical Science (MAZUMS). To enter the patients into the study, we made a study frame of TDT cases who were volunteers to use amlodipine. Based on inclusion criteria, recruiting candidates were randomly operated. A random list was constructed to allocate eligible patients to two sequences. An online web randomizer was applied to generate the random list containing unique codes for the block randomization in this study (https://www.sealedenvelope.com/simple-randomiser/v1/lists). Subsequently, amlodipine and placebo were packaged and then labeled with unique codes. Based on the generated random list, the bottles containing amlodipine or placebo were delivered to the included patients. The outcome assessor, patients, health care providers, and laboratory staff were blind to the study sequences. All placebo tablets were provided by the faculty of pharmacy at Mazandaran University of Medical Science (MAZUMS). The placebo tablets had all compounds except the active ingredient, magnesium stearate, and Avicel, as fillers without biological effects. The placebo tablets were indistinguishable from amlodipine tablets in size, color, and shape.

2.5 | Baseline and clinical data collection

A form comprising demographic information (age, gender, and weight), history of splenectomy, history of diabetes mellitus, transfusion data (duration and amount of red-cell transfusion), iron-chelating therapy (type, dose, and duration), the mean of hemoglobin and ferritin over the last 1 year (g/dl) and the grade of myocardial hemosiderosis was completed for each subject. Moreover, systolic and diastolic blood

pressures (SBPs and DBPs) were also recorded by nurses in the thalassemia ward to monitor the patients' blood pressure. The systolic and diastolic blood pressures measured at the beginning of the study and the end of phases I and II were registered in the datasheet.

2.6 | Specimen collection and analysis

Oxidative stress biomarkers constituted malondialdehyde (MDA), carbonyl protein (protein CO), glutathione (GSH), and total antioxidant capacity (TAC)[18] were measured by in-vitro analyses.[18]

2.7 | Primary outcomes

Oxidative stress indices, including MDA, protein CO, GSH, and TAC, were considered primary outcomes, and blood pressure was the secondary outcome.

2.8 | Malondialdehyde (MDA)

Thiobarbituric acid (TBA) method was operated to value malondialdehyde (MDA) concentrations in the plasma, according to the formation of red (pink) chromophores after the interaction of TBA, MDA, and other breakdown products of peroxidized lipids, which are termed TBA-reactive substances (TBARS). Initially, 200 µl of the standards and samples were poured into the microtube, and 200 µl of 2 M phosphoric acid was also added. Next, 25 µl of TBA reagent was added and placed in boiling Ben Marie for 5 min. After that, they were set on ice to cool completely, and then 500 µl of n-butanol was added to all the microtubes and centrifuged at 160 *g* for 3 min. The absorbance of the samples was measured at 535 nm with an ELISA reader.[19]

2.9 | Carbonyl protein (Protein CO)

To measure carbonyl protein groups, its derivation with DNPH, which leads to the foundation of a stable product (DNP), was used. 200 µl of serum was added to two microtubes (one for the sample and one for the control). Then 500 µl of TCA solution was added to each microtube, kept at 4 ° C for 15 min, and centrifuged at 4700 *g* for 10 min. The supernatant was discarded, and the residue was dispersed in 500 µl of 0.1 M NaOH. Then 500 µl of the DNPH 10 mM (2,4-dinitrophenylhydrazine) reagent was added to the sample microtubes, and 500 µl HCl 2 M was added to the control microtubes. The microtubes were incubated with foil for 30 min at room temperature and in a dark place. Next, 500 µl of TCA solution was added to each microtube and then centrifuged at 4700 *g* for 10 min. After centrifugation, the supernatant was again discarded, and the residue was dispersed with 1000 µl of ethanol and ethyl acetate solution (a ratio of 50–50). Then it was centrifuged again at 4700 *g* for 10 min, and the supernatant was discarded. The residue was dispersed with 200 µl guanine hydrochloride 6 M and absorbed at 405 nm with an ELISA reader. The extinction coefficient for carbonyl groups is 22,300 1-1 cm-M.[20]

2.10 | Glutathione (GSH)

The GSH assay was fulfilled by reacting it with DTNB 5, 5′-dithiobis-2-nitrobenzoic acid (Ellman's reaction). 1.5 ml of TCA was added to 1 ml of diluted serum and centrifuged at 3500 rpm for 15 min after vortex. Then 1 ml of clear supernatant and 1 ml of standards were removed, and 2.5 ml of Tris buffer and 0.5 ml of DTNB reagent were added and incubated for 15 min after the vortex. Finally, the absorbance of the samples at 405 nm was read by an ELISA reader. Glutathione concentration was calculated from the standard curve of glutathione in nmol/ml.[21]

2.11 | Total antioxidant capacity (TAC)

The ferric reducing/antioxidant power (FRAP) test was used to measure total antioxidant capacity. In this test, the antioxidant agents in the sample decreased the ferric tripyridyltriazine (TPTZ-Fe^{3+}) complex to the ferrous (TPTZ-Fe^{2+}) complex, which is blue in the acidic medium. To prepare the FRAP solution, 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution (Sigma–Aldrich) in 40 mM HC1, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 were mixed together. Then, the FRAP solution was heated to 37°C. The 300 µl of FRAP solution and the 10 µl of each standard solution ($FeSO_4 \cdot 7H_2O$) or plasma sample were mixed. The optical absorption of the samples was measured by spectrophotometer at 593 nm against the blanks (distilled water), and the FRAP content of the unknown sample was calculated as compared to the standard curve in Fe^{2+} (µM per liter).[22]

2.12 | Myocardial magnetic resonance imaging T2*

A non-contrast MRI agent was carried out on a 1.5 T MR scanner (Symphony; Siemens, Germany) to value myocardial MRI T2* for all included patients. Myocardial MRI was performed by the Brompton protocol.[23] To calculate myocardial MRI T2*, scans were harmonized to the cardiac cycle via standard electrocardiography gating. A standard radiofrequency body coil was also engaged in all measurements. The region of interest (ROI) was determined in the midventricle. Moderate to severe cardiac siderosis was also itemized according to myocardial MRI T2* value as <14 ms.[24]

2.13 | Statistical analysis

Data have been presented as mean (standard deviation) or number (percentage). The Shapiro–Wilk test and histogram were applied to check the normal distribution for quantitative variables. For numerical

and dichotomous variables, the Student's *t*-test or the Mann–Whitney U-test alongside the chi-square or Fisher's exact test was run to compare the baseline characteristics between the two sequences. An analysis of variance (ANOVA) / analysis of covariance (ANCOVA) was operated after controlling outcome values at the baseline to compute an adjusted effect size. The effect sizes estimated in the study were Hedges's g (with 95% confidence interval [CI]) and eta squared. After transforming eta squared through an online calculator (https://www.psychometrica.de/effect_size.html), a number needed to treat (NNT) was also calculated. A sub-group analysis was also performed based on severe iron overload (ferritin 2500 ng/ml or more).[25] Data analyses were performed by STATA version 13 (StataCorp). $p < 0.05$ was the statistical significance threshold for all two-tailed statistical tests. This study considered a minimum change difference of five percent for each outcome between amlodipine and placebo as clinical significance. We used the website of biorender.com to illustrate the graphical abstract. Ultimately, a power estimation was implemented. The power (1−β) was computed between 0.07 based on protein CO values up to 0.50 according to MDA values (power = 0.20 for GSH and power = 0.35 for TAC). To calculate the study power, the XSAMPSI command was operated in case the MDA, protein CO, GSH, and TAC were considered the dependent variables, type I error = 0.05, and sample size = 17.

3 | RESULTS

Sixty-four patients were evaluated for meeting the inclusion criteria. Of whom, 44 refused to participate in the study, and one patient did not meet the eligibility criteria. Of the 19 patients randomized in the two-period crossover trial, two patients from the AP sequence left the trial and became unwilling to continue the study. At the end of the study, 17 patients completed the trial and were included in the analysis, nine cases from the PA sequence and eight cases from the AP sequence (Figure 1). All patients were under combination therapy with deferoxamine and deferiprone. The number of cases with severe iron overload (ferritin ≥ 2500 ng/ml) was nine (53%). Demographic and clinical characteristics between two sequences were not significant (Table 1).

3.1 | Outcomes: MDA

At the baseline, MDA levels in the AP and PA sequences were 18.17 ± 8.01 μmoL/L and 15.38 ± 2.69 μmoL/L, respectively. The value was reduced to 14.39 ± 1.98 μmoL/L after amlodipine in the AP sequence (mean difference −3.78 μmoL/L). In this sequence, MDA levels after placebo decreased to 15.39 ± 3.57 μmoL/L (mean difference −2.78 μmoL/L). In the PA sequence, the MDA concentration reached 11.48 ± 1.52 μmoL/L and 13.33 ± 2.40 μmoL/L after amlodipine and placebo, respectively (mean difference −3.9 μmoL/L versus mean difference − 2.05 μmoL/L, respectively).

In all cases, there was no significant difference in the decreased values of MDA after a comparison between amlodipine and placebo at the end of the study ($p = 0.17$). The value of MDA lessened from 16.86 ± 6.11 to 13.02 ± 2.28 μmoL/L after amlodipine therapy at the end of the study. A drop in MDA values was also found

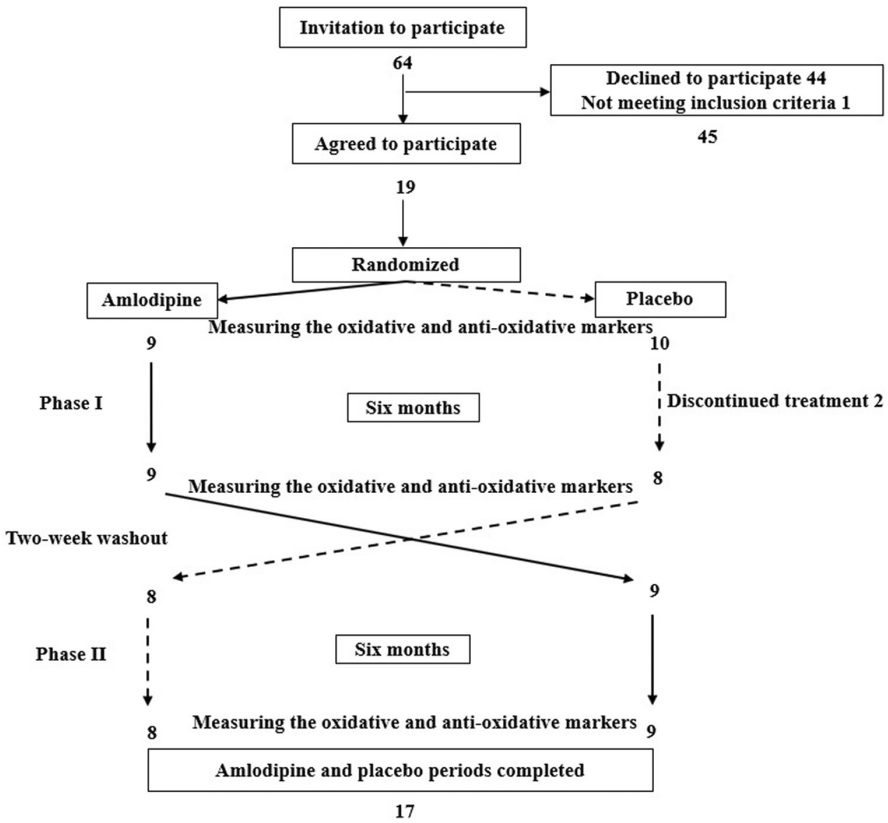

FIGURE 1 Study flowchart

TABLE 1 Basic demographic and clinical characteristics of two sequences.

Variables	Sequence AP ($n = 9$)	Sequence PA ($n = 8$)	p value
Age, year	28.4 ± 5.9	28 ± 6.9	0.59
Gender, M/F ratio	7/2	4/4	0.23
Weight, kg	53.67 ± 6.7	56.5 ± 10.2	0.44
Splenectomy	5 (55.6%)	4 (50%)	0.81
Time since splenectomy, year	11.8 ± 5.6	19.75 ± 6.9	0.17
DM	3 (33.3%)	2 (25%)	0.7
Duration of DM, year	5.06 ± 6.1	6 ± 5.6	1
Length of red-cell transfusion, year	24.8 ± 5.36	26.1 ± 5.98	0.96
Amount of red-cell transfusion, cc/kg/month	16.8 ± 3.6	16.4 ± 6.5	0.44
Duration of DFO therapy (year)	20.9 ± 9.1	21.3 ± 3	0.53
Dose of DFO therapy (mg/day)	1777 ± 263	1750 ± 378	0.86
Duration of DFP therapy, year	7.1 ± 5.2	8.9 ± 4.8	0.56
Dose of DFP therapy (mg/day)	3055 ± 167	2812 ± 530	0.21
Duration of combination therapy with DFO and DFP, year	6.2 ± 4.5	8.9 ± 4.8	0.26
Duration of iron chelation therapy (totally), year	22.6 ± 6.4	21.8 ± 2.6	0.62
Hemoglobin, g/dl	8.33 ± 0.5	8.9 ± 0.8	0.80
Ferritin (ng/ml)	3420 ± 2317	2958 ± 2335	0.69
Myocardial MRI T2*(ms)	10.7 ± 2.8	8.9 ± 2.2	0.18
Moderate cardiac hemosiderosis	5 (55.5)	3 (37.5)	0.64
Severe cardiac hemosiderosis	4 (44.5)	5 (62.5)	0.64

Note: Data presented as number (percentage) or mean ± standard deviation.

Abbreviations: DFO, deferoxamine; DFP, deferiprone; DM, diabetes mellitus; ms, millisecond; myocardial MRI T2*, myocardial T2*-weighted magnetic resonance imaging; Sequence AP, amlodipine first and then placebo; Sequence PA, placebo first and then amlodipine.

following placebo (16.86 ± 6.11 to 14.42 ± 3.17 μmoL/L) (Table 2; Figure 2). The result of the subgroup analysis displayed that all included patients with severe iron overload (ferritin ≥ 2500 ng/ml) treated with amlodipine experienced a decline in MAD levels from 14.47 ± 3.30 μmoL/L to 12.83 ± 4.31 μmoL/L. In this sub-group, the MAD level following the placebo was 14.07 ± 4.31 μmoL/L ($p = 0.41$).

3.2 | Protein CO

The protein CO levels at the baseline were 1.71 ± 0.22 μM/L and 1.65 ± 0.24 μM/L in both sequences of the study. In the AP sequence, a drop in protein CO level was found after amlodipine and placebo, 1.58 ± 0.34 μM/L (mean difference − 0.13 μM/L) and 1.60 ± 0.54 μM/L (mean difference − 0.11 μM/L), respectively. In the PA sequence, protein CO levels decreased to 1.60 ± 0.28 μM/L after amlodipine (mean difference − 0.05). The values of protein CO after the placebo remained unchanged in this sequence. In all cases, compared to the placebo, amlodipine led to a non-significant decrease in protein CO concentrations ($p = 0.84$). The baseline level of protein CO was 1.68 ± 0.22 μM/L. The concentration of protein CO decreased to 1.59 ± 0.43 μM/L after amlodipine compared to 1.63 ± 0.27 μM/L after placebo (Table 2 and Figure 2). In severe iron-overloaded cases (ferritin ≥ 2500 ng/ml), the value of protein CO was 1.68 ± 0.19 μM/L at the baseline. The values of protein CO after amlodipine and placebo were 1.56 ± 0.46 μM/L and 1.61 ± 0.46 μM/L, respectively ($p = 0.1$).

3.3 | GSH

At the baseline, serum GSH concentrations were 146.6 ± 14.86 nM/L and 146.1 ± 19.00 nM/L in both study sequences. Increased serum GSH concentrations in the AP sequence were detected following amlodipine, 153.8 ± 18.05 nM/L (mean difference + 7.2 nM/L). However, the value remained unchanged after the placebo in this sequence. In the PA sequence, serum GSH concentrations rose to 155.03 ± 16.86 nM/L and 153.1 ± 9.29 nM/L after amlodipine and placebo, respectively (mean difference + 8.93 nM/L versus mean difference + 7 nM/L, correspondingly).

In all cases, at the end of the study, a non-significant increase in serum GSH concentration was observed after amlodipine compared to placebo ($p = 0.88$). In all cases, the level of GSH was 146.4 ± 16.39 nM/L at the baseline. After amlodipine, the mean of

TABLE 2 Changes of oxidative and antioxidative markers (crude model).

	All patients ($n = 17$)				Sequence AP ($n = 9$)				Sequence PA ($n = 8$)			
	Baseline	After Amlodipine	After Placebo	*p* value	Baseline	After Amlodipine	After Placebo	*p* value	Baseline	After Amlodipine	After Placebo	*p* value
MDA, μmol/L												
Mean	16.86	13.02	14.42	0.15	18.17	14.39	15.39	0.47	15.38	11.48	13.33	0.09
SD	6.11	2.28	3.17		8.01	1.98	3.57		2.69	1.52	2.40	
Protein CO, μM/L												
Mean	1.68	1.59	1.63	0.75	1.71	1.58	1.60	0.87	1.65	1.60	1.65	0.80
SD	0.22	0.43	0.27		0.22	0.34	0.28		0.24	0.54	0.27	
GSH, nM/L												
Mean	146.4	154.4	149.6	0.88	146.6	153.8	146.5	0.48	146.1	155.03	153.1	0.79
SD	16.39	16.96	19.19		14.86	18.05	25.24		19.00	16.86	9.29	
TAC, μmol FeSO4/L												
Mean	55.71	64.26	57.03	0.26	60.32	73.86	61.59	0.24	50.53	53.45	51.90	0.75
SD	13.26	20.21	16.15		14.43	22.14	20.49		10.29	11.04	7.73	

Note: *p* value for after amlodipine and placebo, Student's t-test for comparing oxidative antioxidative markers.

Abbreviations: GSH, glutathione; MDA, malondialdehyde; Protein CO, protein carbonyl; SD, standard deviation; Sequence AP, amlodipine first and then placebo; Sequence PA, placebo first and then amlodipine; TAC, total antioxidant capacity.

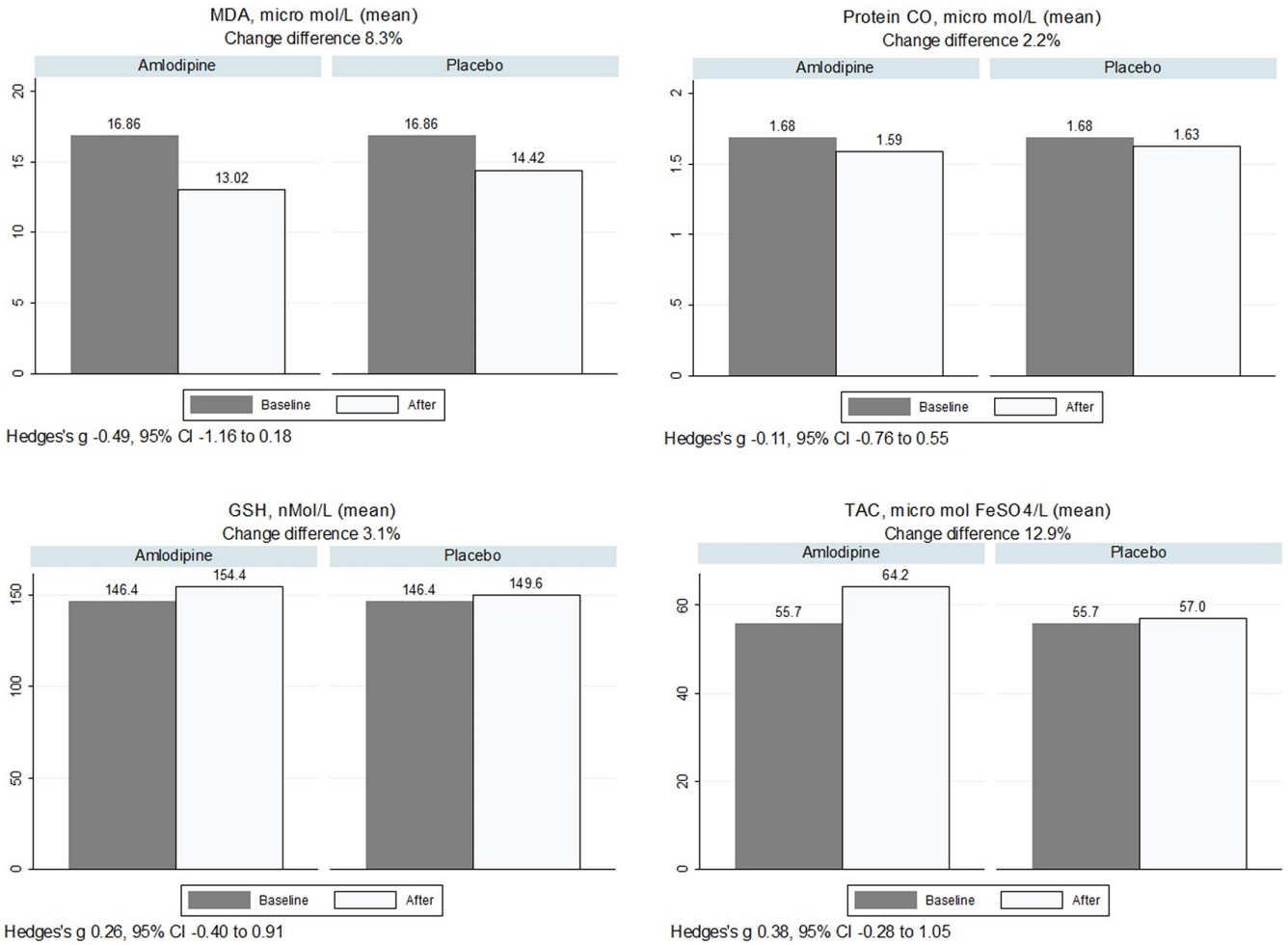

FIGURE 2 The change difference (%) for each outcome between amlodipine and placebo. Hedges's g (95% CI) was estimated for changes in oxidative and anti-oxidative markers (crude model). GSH, glutathione; MDA, malondialdehyde; Protein CO, protein carbonyl; TAC, total antioxidant capacity.

GSH rose to 154.4 ± 16.96 nM/L. The mean of GSH increased to 149.6 ± 19.19 nM/L after the placebo (Table 2 and Figure 2). At the baseline, the GSH level in the patients with severe iron overload (ferritin ≥ 2500 ng/ml) was 148.83 ± 12.75 nM/L. After treatment with amlodipine, the levels of GSH reached 159.8 ± 24.48 nM/L, while the value changed to 155.9 ± 24.48 nM/L after placebo ($p = 0.65$).

3.4 | TAC

At the baseline, serum TAC levels in the sequences of AP and PA were 60.32 ± 14.43 μmol FeSO4/L and 50.53 ± 10.29 μmol FeSO4/L, respectively. The values rose to 73.86 ± 22.14 μmol FeSO4/L (mean difference + 13.54 μmol FeSO4/L) after amlodipine and to 61.59 ± 20.49 μmol FeSO4/L (mean difference + 1.27 μmol FeSO4/L) after placebo in the AP sequence. In the PA sequence, the levels changed to 53.45 ± 11.04 μmol FeSO4/L and 51.90 ± 7.73 μmol FeSO4/L after amlodipine and placebo, respectively (mean difference + 2.96 μmol FeSO4/L versus mean difference + 1.37 μmol FeSO4/L, correspondingly).

In all cases, the mean serum TAC levels in those under treatment with amlodipine non-significantly increased compared to placebo receivers ($p = 0.26$). At the end of the study, serum TAC levels scaled up from 55.71 ± 13.26 μmol FeSO4/L to 64.26 ± 20.21 μmol FeSO4/L following amlodipine treatment. The change of TAC after the placebo witnessed an increase at 57.03 ± 16.15 μmol FeSO4/L (Table 2 and Figure 2). The patients with severe iron overload (ferritin ≥ 2500 ng/ml) had TAC levels of 57.51 ± 12.72 μmol FeSO4/L at the beginning of the study. There was an increase in TAC level after amlodipine (68.31 ± 30.05 μmol FeSO4/L). After the placebo, the TAC level changed to 63.70 ± 30.05 μmol FeSO4/L ($p = 0.66$). According to the study sequences, the values of oxidative and anti-oxidative indices have been presented in Table 2.

The outcomes at the end of the study discovered non-significant differences for the oxidative and anti-oxidative indices after controlling the measurements of MDA, protein CO, GSH, and TAC at the baseline ($p = 0.09$, $p = 0.76$, $p = 0.45$, and $p = 0.21$, respectively). The Hedges's g for MDA adjusted based on MDA values at the baseline was estimated at −0.59, 95% CI −1.26 to 0.08. After controlling the baseline protein CO values, Hedges's g was computed at −0.11,

95% CI −0.76 to 0.55. The adjusted values of Hedges's g equal to 0.26, 95% CI −0.40 to 0.91 and 0.42, 95% CI −0.24 to 1.09 were also approximated for GSH and TAC, respectively. The current study estimated the NNTs for MDA, protein CO, GSH, and TAC, which were 4, 17, 7, and 5, correspondingly. In addition, the change difference for MDA was 8.3% (protein CO 2.2%, GSH 3.1%, and TAC 12.9%), representing the value for MDA and TAC was clinically significant (Table 3).

3.5 | Blood pressure

During the study, no adverse effects were founded. The mean SBP at the baseline and after amlodipine and placebo were 99.4 ± 5.56 mmHg, 98.3 ± 5.14 mmHg, and 101.6 ± 6.88 mmHg, respectively. The means of DBP were also 65.3 ± 6.24 mmHg at the baseline, 70 ± 7.67 mmHg, and 69.5 ± 6.21 mmHg after amlodipine and placebo, respectively.

4 | DISCUSSION

The study findings discovered that amlodipine therapy for 6 months accomplished by standard routine treatment with iron chelators could apply a decent impact on MDA and TAC concentrations amid iron-overloaded TDT cases. Around five, an optimum approximated NNT for adjuvant therapy with amlodipine was found for MDA and TAC in this study, introducing its clinical merit for clinicians to help normalize these components efficiently. However, the effect of amlodipine on the levels of protein CO and GSH was weak. Amlodipine treatment could change the levels of MDA and TAC to the extent of clinical significance defined for this trial in the context of off-label use of the drug. In the current study, no adverse side effects were detected in parallel to other studies.[26,27]

Amlodipine might improve antioxidant status by reducing the uptake of non-transferrin-bound iron (NTBI), mitigating oxidative stress.[28] The clinical evidence regarding the antioxidant effects of amlodipine in β-thalassemia patients is limited. Some studies have assessed that amlodipine as a calcium channel blocker (CCB) can be efficient in iron overload circumstances. A 12-month trial, including 221 hypertensive cases, showed that myeloperoxidase (MPO) as an oxidative stress index was significantly mitigated after monotherapy with amlodipine 10 mg (Hedges's g 0.42, 95% CI 0.09 to 0.75). However, the concentrations of lipoprotein (a), paraoxonase-1 (PON-1), and isoprostanes did not noticeably change.[29] Systolic blood pressure significantly reduced after the monotherapy, compared to the baseline values (128 ± 5 mmHg versus 149 ± 7 mmHg, respectively).

It has been unknown exactly how amlodipine exerts its antioxidant activity. In-vitro studies have revealed that CCBs may apply antioxidant properties via multiple pathways and plausible mechanisms, including antithrombotic and anti-inflammatory effects and a decrease in plasma low-density lipoprotein (LDL) cholesterol

TABLE 3 MDA, protein CO, GSH, and TAC distributions after amlodipine and placebo (adjusted model).

Model		Time point	Amlodipine $n = 17$	Placebo $n = 17$	Change difference, %	Hedges's g (95% CI)	NNT	Eta squared	Adjusted R^2	F statistic	p value
Adjusted	MDA, μmol/L	Post	13.02 ± 2.30	14.42 ± 2.30	8.3	−0.59 (−1.26 to 0.08)	4	0.06	0.33	3.14	0.09
	Protein CO, μM/L	Post	1.59 ± 0.36	1.63 ± 0.36	2.2	−0.11 (−0.76 to 0.55)	17	0.003	−0.04	0.10	0.76
	GSH, nM/L	Post	154.4 ± 18.23	149.6 ± 18.23	3.1	0.26 (−0.40 to 0.91)	7	0.02	−0.02	0.58	0.45
	TAC, μmol FeSO4/L	Post	64.26 ± 16.58	57.03 ± 16.58	12.9	0.42 (−0.24 to 1.09)	5	0.04	0.19	1.62	0.21

Note: Adjusted for the outcomes measured at the baseline (calculated based on ANOVA/ANCOVA model). Change difference was calculated for each outcome between amlodipine and placebo based on the baseline values. Data presented as number (percentage) or mean ± standard deviation.

Abbreviations: CI, confidence interval; GSH, glutathione; MDA, malondialdehyde; NNT, number needed to treat; Protein CO, protein carbonyl; TAC, total antioxidant capacity.

levels. These L-type CCBs may have antioxidant properties due to their chemical structure, including an aromatic ring that attracts free radicals. In addition, the dihydropyridine ring in these CCBs will donate a proton, stabilizing the free electron.[30] Amlodipine may impede calcium influx, the leading mechanism to suppress oxidative stress. CCBs can impact the cellular interaction of endothelial cells, smooth muscle cells, monocytes, and thrombocytes, which play pivotal parts in the initial stages of atherosclerosis growth. Consequently, the prevention of intercellular calcium overload is one of the underlying mechanisms that amlodipine applies to its antioxidant impact.[31,32]

After inhibiting low voltage-dependent calcium channel (LVDCC) with amlodipine, iron levels alongside oxidative stress in myocardial cells will be mitigated, improving patients' clinical outcomes and even their survival.[33,34] The results of an animal investigation uncovered that amlodipine could reduce oxidative stress by inhibiting excessive MDA production. According to the findings, amlodipine may reduce oxidative stress by strengthening the glutathione system.[35] In an experimental study on 42 diazinon-poisoned rats with increased oxidative stress, the administration of amlodipine could ameliorate lipid peroxidation levels and raise TAC levels.[36] Amlodipine might impose its antioxidant properties by blocking calcium channels and regulating intracellular calcium. Amlodipine would be a protective factor that prevents free radical generation from oxidative damage.[36]

Appropriately, it is hypothesized that amlodipine may inhibit oxidative stress by impeding iron uptake. A study's results with a standard animal model of iron surplus and cardiomyopathy demonstrated that treatment with amlodipine—2.5 mg orally for 5 days per week for 4 weeks — could be comparatively effective in decreasing iron uptake and oxygen-free radical formation in the cardiac among iron-overloaded mice.[28] Another study revealed that the prescription of amlodipine—as a lipophilic dihydropyridine CCBs — has a protective property against oxidative stress via the inhibition of noxious ROS production and is likely to be advantageous for patients with iron overload status.[37] In the aforementioned study, 75% and 25% mortality rates were reported in non-amlodipine users and the subjects treated with amlodipine, respectively.

5 | CONCLUSION

Since iron-overloaded β-thalassemia patients suffer from high levels of oxidative stress, which is one of the paramount causes of morbidities in this population, considering amlodipine as an adjuvant alongside standard treatment can be advantageous. Clinically, amlodipine added to conventional iron chelators is an efficient therapy for improving the levels of MDA and TAC in patients with TDT. However, more clinical trials are desirable to verify the results. In the current trial, we had some shortcomings, comprising the small number of volunteers and the short length of amlodipine therapy. As such, in future, a study of more than 6 months for the off-label use of amlodipine is more likely to bring better results on the oxidation level of proteins in β-thalassemia patients. We also suggest reproducing the present research with a larger sample size involved in multi-centers to enhance the power of the study and generalizability. Furthermore, compliance and adherence to amlodipine/placebo were not evaluated in this study. It should be mentioned that our results need corroboration with other thalassemic populations because of the potential source of bias arising from the off-label use of amlodipine.

AUTHOR CONTRIBUTIONS

E.S and H.D.K.H involved in the study concept and design; H.K.A, E.S, and H.K involved in search of the database; A.A and M.Z involved in the acquisition of data; F.S.H, H.K.A, and R.N.A involved in antioxidant biomarker measurements; H.D.K.H involved in data analysis; R.N.A, A.A, and M.Z involved in drafting of the article; H.D.K.H, H.K, M.K, and E.S involved in interpretation of data or critical revision of the article. All authors verified the article's content and certified the accuracy or integrity of any part of the study.

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CONFLICT OF INTEREST

No conflict of interest exists for any authors associated with the article. There was no source of extra-institutional commercial funding as well.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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